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Effect of A Moderate Aerobic Exercise Training Program on Ovarian Function

There is substantial evidence to suggest that estrogens play a key role in the etiology of breast cancer. Both cross-sectional studies of highly trained athletes and prospective studies of high intensity exercise training programs have found a higher frequency of anovulation, lower levels of estradiol and in some cases a shortened luteal phase length with associated lower estradiol levels among these women. However, little is known about the effects of *moderate intensity* exercise on ovarian function. The aim of this study was to investigate the relationship between a *moderate intensity* exercise training program and ovarian function. Specifically, we aimed: 1) to determine whether changes occurred in frequency of ovulation as a result of a 6 month exercise training program, 2) to determine whether changes occurred in serum E_2 levels in ovulatory and anovulatory cycles in these women, and 3) to determine the luteal phase menstrual cycle lengths of these women as a result of the training program. We have completed data collection on 58 women. The analysis of this study will be completed as soon as the hormone analyses are completed.

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Kelly K. Peters 10/12/99

PI - Signature Date

Table of Contents

The Effect of a Moderate Aerobic Exercise Training Program on Ovarian Function ('Final' Annual Report)

Section 5: INTRODUCTION

Breast cancer is the most common serious cancer occurring in American women. As a cause of death among women, breast cancer ranks second only to lung cancer [1]. On the basis of current incidence rates, one in nine women will be diagnosed with breast cancer in her lifetime [1].

There is substantial experimental, clinical and epidemiological evidence that ovarian hormones, particularly estrogens, play a major role in breast cancer risk. Studies have shown that lower levels of estrogen are associated with a reduced risk of disease [2,3].

The study described here will generate new information about the influence of exercise on ovarian function in non-athletes. By beginning the process of establishing how much exercise is needed to reduce estrogen levels, we hope to be able to provide practical advice to women on how to reduce their breast cancer risk.

Hormones and breast cancer risk:

A great deal of evidence exists demonstrating that ovarian hormones, in particular estrogens, play a major role in breast cancer risk [2,3]. The age-incidence relationship of the common non-hormone related cancers such as stomach and bladder shows a continuous steady increase with age. In contrast, breast cancer incidence increases steadily and rapidly with age until about age 50 (average age at menopause) at which time the rate of increase slows dramatically [2]. Direct epidemiological study of the effect of age at menopause shows that for each year a woman's ovaries continue to function there is a 10% increase in her subsequent breast cancer risk [1,2,4]; this is true whether the menopause is natural or artificial (bilateral oophorectomy). The decline in the rate of increase in incidence around age 50 is thus directly correlated with the markedly reduced serum levels of estrogen (and progesterone) after menopause.

Ovulating women in low breast cancer risk Asian countries have been shown to have lower levels of circulating estrogens than women in the US and the UK, both high risk countries [5]. Postmenopausal breast cancer cases have been found to have higher serum estrogen levels than controls [3]. Studies, which paid strict attention to factors which may influence hormone levels in cases, found statistically significant elevated serum levels of estradiol in premenopausal breast cases compared to controls [5].

Estrogen is presumed to increase risk of breast cancer through its known action as a breast cell mitogen [2]. Higher levels of endogenous estrogen would be expected to increase mitotic activity. Both follicular and luteal phase estradiol (E2) are of interest; the breast cell proliferation rate in the follicular phase is some 50% that in the luteal phase and so E2 levels in both phases are important [2]. Progesterone (Prg) also acts to increase breast cell proliferation. We are making measurements of both E2 and Prg.

Exercise and breast cancer risk:

A survey of surviving Harvard female college athletes found that the athletes had a 46% reduction in prevalence of breast cancer compared to non-athletes (24/2622 vs. 45/2776; 2 sided P=0.05) [6]. A Finnish cohort study showed that physical education teachers had a 19% lower risk of breast cancer than language teachers, but the results were not statistically significant (22/924 vs. 106/3239; 2-sided P=0.21) [7]. The NHANES I cohort was reported as showing no overall relationship between exercise level and breast cancer risk, but the questions asking about exercise activity had no duration component and the study has to be considered non-informative [8].

We completed a case-control study of 545 young (age 40 or younger) breast cancer cases and 545 control women matched for age, race, parity and neighborhood of residence [9]. The

daily average lifetime (post menarche) number of hours spent in exercise activities was a significant predictor of reduced breast cancer risk (2-sided P<0.0001). Compared to inactive women, risk of breast cancer was reduced by 27% in women who exercised on average 2.5 hours per week, and was reduced by 58% in women who exercising 4 or more hours per week (average approximately 60 mins/day).

Exercise and reproductive function:

We believe, based on our understanding of the relation of ovarian hormones to breast cancer risk [2,3], that the observed protective effect of exercise against breast cancer is likely to be due to a reduction in exposure to serum estrogen. Reduced serum estrogen levels may be due to an increased frequency of anovulatory cycles and/or to decreased circulating levels of estrogen in ovulatory cycles. E2 is the most important estrogen and we have concentrated our attention on E2 in this study [2,3]. Cycles with long follicular phase are associated with lower than average cumulative E2 exposure since such cycles have an increased number of days with early follicular phase low E2 levels. Cycles with short luteal phases have also been found to be associated with low E2 values [10].

Training studies:

The studies discussed above compared groups of women who were self selected on exercise level. Some or all of the effects may, therefore, be due to other aspects of their lifestyles or to genetic factors that are strongly correlated with exercise activity. Furthermore, the validity of self-reported responses in cross-sectional studies are of concern. The strength of this prospective training study lies in the ability to structure and monitor the type, intensity and duration of exercise without having to rely on second-hand reports.

Except for a study by Shangold et al. [11] of a single individual, we have identified only four exercise training studies of exercise and ovarian function under a controlled protocol which included pre- and post-training testing, all were conducted using very few subjects. The first of these studies was conducted by Boyden et al. [12] among 19 women who had previously engaged in informal running (average, 15.1 mi/wk) and were trained rigorously to run a full marathon over a 14 to 15 month period. Each subject had blood samples taken at baseline, after their weekly mileage had increased by 30 mi/wk and again when weekly mileage had increased by 50 mi/wk. The mean plasma mid-follicular E2 values were 76% of the baseline values after a weekly mileage increase of 30 mi/wk and 48% at 50 mi/wk (2-sided P=0.03). However, Prg values were not obtained during the test cycles, and it is, therefore, unclear as to whether the E2 values reported occurred within ovulatory cycles.

In a study by Bullen et al. [13], 7 young women with prior athletic experience were trained at a high-intensity. The training protocol consisted of cycle ergometry 2 days/wk and running 4 days/wk at exercise intensities eliciting 85% of maximum heart rate. The duration of high-intensity activity was increased from 20 mins/session to 45 mins/session over a 4 week period. Subjects trained at 45 mins/session for the remaining 4 weeks of the study. All of the cycles appeared to have been ovulatory, as evidenced by midcycle surges of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Diminished urinary estriol (E3) levels were observed in 4 of the 7 subjects. Serum E2 levels were reported to have not changed appreciably, however no quantitative values were offered in the published report.

In a subsequent study of Bullen et al. [14], 28 initially untrained women with documented ovulation (urinary LH surge) were studied to determine whether strenuous exercise spanning two menstrual cycles would induce menstrual disorders. Initially, subjects ran 4 mi/day and increased their training regimen to 10 mi/day by the end of week 5 and continued to run 10 mi/day for the remaining 5 weeks of the study. Subjects ran at 75% to 80% of maximum heart rate. Only 4 of 28 subjects (14%) had a normal cycle during one or both periods of exercise training. The criterion for normalcy included "a biphasic temperature curve, an

ovulatory pattern of changes in gonadotropin and sex steroid excretion, and normal luteal function, defined as excretion of free Prg in a characteristic parabolic curve between the LH surge and beginning of the following menses". Keizer et al. [15] assessed the effect of a 12 week endurance training program on plasma hormone responses among 8 previously untrained women. The training program consisted of running (2-3 times/week) and cycling (once/week). The training duration and intensity was progressively increased from a mean running speed of 9 km/hr (approx. 60% VO_2 max, equivalent to 65% of maximum heart rate [16]) and 20 min/day to 11-12 km/hr (approx. 80-85% of maximum heart rate) and 50-75 min/day. After training, follicular E2 values were 58% higher. However, the mean E2 value in the luteal phase was 57% (2-sided $P<0.01$) of the pre-training value. All subjects were reported to have ovulated in the pre-training test cycle. Based on pre- and post-training levels of Prg in the luteal phase (22.3 +/- 4.9 nmol/l, 20.8 +/- 0.4 nmol, respectively), all subjects were reported to have ovulated post-training, however, individual values were not reported and the data remain inconclusive. It is unclear when post-training measurements were obtained.

In summary, there is some evidence to suggest that high intensity exercise training programs alter ovarian function and are in agreement with cross-sectional studies. There have been no reported studies which assess the effect of a moderate exercise training program on ovarian function in previously sedentary women [16].

In this study, we have conducted a modified version of the above described studies by integrating elements of each study to meet our standards of a moderate intensity exercise training program in which previously inactive women can reasonably be expected to partake in. We have enrolled subjects in a 6 month moderate exercise training program. Developing a long-term exercise training program allows us to assess the chronic effects of a moderate intensity exercise program on ovarian function. Furthermore, we have a substantially larger sample size than in other training studies. We are able to assess the specific effects of aerobic activity with changes in E2 and Prg. In addition to determining changes in ovarian hormone levels, we will assess changes in luteal phase length and frequency of anovulation. These latter parameters have not been assessed in a prospective training study and will add significantly to our understanding of the effects of *moderate* exercise on ovarian function.

Section 6: BODY

Hypotheses

The hypotheses of this study are:

1) Frequency of ovulation will be reduced as a result of a 6 month aerobic exercise training program of moderate intensity.
2) Serum estradiol (E2) levels will be lower as a result of a 6 month aerobic exercise training program of moderate intensity.
3) Luteal phase menstrual cycle lengths will be shorter as a result of a 6 month aerobic exercise training program of moderate intensity.

Procedures

A prospective study has been undertaken to assess the effect of a 6 month moderate intensity exercise training program on basal hormonal levels among previously sedentary premenopausal women. In this study, we have collected blood and urine specimens (baseline, after 14 weeks on the training program and near the end of the 6 month training program) and questionnaire data from women who agreed to participate in a 6 month exercise training program.

Subject Selection

Interested female participants were asked to complete a brief screening survey. The screening survey is intended to identify subjects who meet our criteria of inclusion. All study participants had to meet the following criteria:

* nulliparous
* 18 to 35 years of age
* free of underlying diseases or conditions that may interfere with the measurement of hormone levels and/or the interpretation of hormone data
* have not used hormonal contraceptives over the past six months and not planning to over the course of participation in the study
* average menstrual cycle length between 15 and 45 days
* no regular exercise over the past 6 months
* BMI value between 20 and 30 kg/m^2
* no dieting over the past 6 months
* no smoking over the past 6 months

These criteria were set to reduce the impact of confounding variables which may be associated with altered ovarian function. We contact all interested women and review the responses provided on the screening survey to confirm eligibility. We then asked selected participants to notify us of the first day of their next menstrual cycle. At that time, subjects were asked to meet with us at a specified location, at a mutually convenient time to sign to an Informed Consent, to pick up a study kit, and to receive the questionnaire (described below).

Training Protocol:

A 6 month endurance training program was undertaken. We chose to conduct this training program over an extended period of time in an effort to determine the effects of a long-term exercise training program of moderate intensity on ovarian function, the effects of which are presently unknown. Subjects reported to the study site and engaged in a monitored aerobic exercise training program for a total of 3 hours per week. The participants begin their training at 50% of their maximum heart rate for 20 minutes per session. Their training regimen is increased gradually to 60 minutes per session while they are at 65% of their maximum heart rate.

Over the last 4 months of the study, each subject exercises for an hour each session at about 65% of her maximum heart rate.

Data collection:

Height and weight and percent body fat (months 1,4 and 7): Subjects were weighed without shoes or over-sweaters. Measurement of height was done without shoes. Body fat was measured using skin calipers at hip, waist and arm.

Questionnaire (month 1): Each participant completed a structured questionnaire. This questionnaire was designed by combining other questionnaires developed by Dr. Bernstein in her previous studies of exercise activity and risk of breast cancer, and of the effect of exercise activity on menstrual patterns in adolescents, as well as questionnaires developed by Dr. Paffenbarger and his colleagues on physical activity [17-19], and Dr. Willett and his colleagues on diet [20-22].

Diet is assessed with a slightly modified version of the Semi-quantitative Food Frequency Questionnaire (SFFQ) developed and validated by Dr. Willett and his colleagues [20-22]. We modified the original questionnaire in consultation with Dr. Willett by adding a list of

24 additional foods commonly consumed in Southern California. The main aim of collecting these data will be to investigate whether diet is a confounder of any effects found with exercise.

Daily records and menstrual calendars (months 1-7): Sedentary subjects were recruited and were requested to avoid making any substantial changes in lifestyle habits (e.g. diet, smoking, drinking and exercise --aside from the training study). Participants were asked to maintain menstrual cycle calendars over the duration of the study. On the calendar, each participant recorded each day of menstrual bleeding for each menstrual cycle that occurs by circling the appropriate dates. These calendars are used to determine menstrual cycle lengths and to monitor menstrual cycle frequency over the duration of the study. Attendance and activity at the gym of each participant was monitored.

Biological collections:

Biological specimens (urine and blood) were collected at baseline (month 1), at midpoint (month 4) and during the final month of training (month 7).

Urine collection: During the collection cycles, daily urine samples beginning on cycle day 10 and continuing until the first day of the next cycle were collected. Plastic bottles for urine collection were provided. Accompanying the bottles was a list of directions instructing the subject to collect a 30 ml sample of first morning urine and specifying procedures to follow. Each participant's progress was monitored. At least once a week, subjects were required to deposit their daily urine specimens at the health club for processing. Samples are picked up daily and stored on the medical campus at -20°C until analyzed.

Serum collection: During the collection cycles, subjects were asked to provide from 2 to 5 15 ml blood samples (depending on the length of their cycle). Blood was taken on cycle days 11 (±1) and 22 (±1) [and subsequently on days 29 (±1), 36 (±1) and 43 (±1), in the event menses has not occurred]. Most subjects need to provide only 2 or 3 samples. Allowance was made for a one day variation to account for samples which may fall on a weekend and for unavoidable conflicts. Subjects must report to the gym between 7:30 AM and 9:30 AM (on the day of their scheduled appointment) in a fasting state and have refrained from exercise activities for at least 5 hours. Blood specimens were processed into serum and stored at -20°C.

Data management:

The day-to-day tracking of participants has been managed on an IBM compatible PC using EXCEL. Using this database, we can effectively monitor participants accrual, assessment responses, gym attendance and track all data collection throughout the study (i.e., questionnaire data, physical measurements, daily physical activity logs, urine and serum collection appointments, etc.).

Both the initial screening questionnaire and the study questionnaire have been coded and entered into an EXCEL file. Height, weight, and body fat measurements have been coded and entered into an EXCEL file as well. The completed Diet Assessments are checked for stray marks and completeness of coding and will be sent to Dr. Willett for analysis [34-36].

We strictly maintain the confidentiality of data through use of locked cabinets accessible only to employees directly involved in the study who have signed an employee confidentiality form. Computer-stored information has only the study identification number to ensure security. We will publish results from the study in tabular descriptions of groups or in a form which precludes identification of specific individuals.

Progress (Year 1):

We devoted an enormous amount of time and effort in year 1 to the advertisement and recruitment for this study. As indicated above, our study criteria are quite rigorous and only very select women meet these criteria.

We began our recruitment efforts by placing fliers within a one mile radius of the designated study sites. We placed fliers at grocery stores, video stores, movie theatres, shopping malls and various other local shops. Additionally, we attended local health fairs and posted signs in several of the larger engineering and computer firms (e.g., Xerox, TRW, Hughes Aircraft, Mattel, Aerospace).

As a study incentive, we arranged to provide a 6 month health club membership at the health clubs at no cost to the subject. At the end of the training study, each participant who had fully completed the study, receives a reduced membership rate to continue use of these facilitates and is compensated with $160.00 for their time, effort and transportation costs.

During this year 59 participants met our criteria and were enrolled in the study. At the end of Year 1, 6 participants had completed the study, 26 were currently exercising, and 7 were in the baseline phase. The remaining 20 participants had dropped out of the study.

Progress (Year 2):

We enhanced our strategy for recruitment during the second year of this study. We began placing ads in the health and calendar sections of local newspapers and cable television stations to increase our range of exposure. We secured spots on 4 area cable stations and 9 newspapers covering a range from as far east as Pasadena traveling west through Santa Monica and downward into the South bay. We were featured in the downtown news, participated in the downtown health fair and spoke to several of the large businesses in the area. We also found it useful to set-up display tables at local health fairs, shopping malls, and other highly visited sites. Several participating subjects suggested this study to friends and colleagues.

Progress (Year 3):

The original protocol called for stationary bicycling only. Due to complaints of boredom from many of the participants, we extended the type of activity to include other forms of aerobic activity such as the treadmill, stairmaster and aerobic classes. We implemented this change with the first exercising subject and have continued to utilize this slightly modified protocol. Frequency, intensity and duration of these activities has not been altered and participants are required to wear a heart monitor to record intensity and duration of activity. To account for vacation or sick leave, we added additional weeks to the program, accordingly, to ensure each subject has participated for 24 weeks.

As outlined in our year 2 progress report, we underestimated the difficulty in recruiting for a 6 month exercise training study in part, due to our rigorous inclusion criteria. Additionally, we reported a 50% drop out rate - higher than the 30% we had expected.
We found that a number of subjects continued to participate through the 3 or 4 month mark and were lost to the study shortly thereafter. We requested IRB approval to conduct a midpoint measurement (defined as the next menstrual cycle beginning after the completion of 14 weeks of exercise). We received IRB approval for this addendum on October 12, 1998 and incorporated this additional 4 month blood draw and urine collection shortly thereafter. The addendum has provided useful information on subjects lost to follow-up over the originally planned 24 weeks.

Progress (Year 4):

We have completed recruitment of 58 women to this study. Fewer than we planned, but this is still a very large study compared to previous work on this difficult topic. Fifty three of these women completed 6 months on study and an additional 5 completed 4 months on study. Seventy seven women who began the exercise program after providing baseline (month 1) blood

and urine samples dropped out of the exercise program before providing any follow-up blood or urine specimens.

The 360 blood samples from the 58 women who provided follow-up blood and urine are currently being measured for E2 and Prg, and the 2550 urine samples will be tested for LH to establish the day of ovulation.

The Effect of a Moderate Aerobic Exercise Training Program on Ovarian Function

Report on Progress of Analysis Phase of Study since Report of September 26, 2000

We recruited 58 women to this study who provided serum samples at baseline ('Baseline') and during the last month of the exercise program ('During Training'). We report here our analysis of the effects of the training regimen on ovulatory frequency and on serum E2 and P4 levels.

Methods

Hormone assays:

Serum E2, P4 and sex hormone binding globulin (SHBG) levels were measured in the laboratory of Dr. Stanczyk. All assays were conducted blindly. Serum E2 was measured using an iodinated (RIA) kit from Pantex (Santa Monica, CA). Serum P4 was measured by RIA after extraction with hexane as described previously [1]. Serum SHBG was measured using an RIA kit from Diagnostics Systems Laboratories (Webster, TX).

Determination of ovulation:

We classified each cycle as ovulatory, anovulatory or indeterminate based on the availability (and value) of a serum P4 on days –3 to –11 (i.e., the availability of a serum P4 between 3 and 11 days before the onset of the next menses). This range of informative days was based on the results presented by Israel and colleagues [2] and inspection of our results (in this and other studies) when we had multiple P4 values. Israel and colleagues suggest that the range of days should be between 4 and 11 days before the onset of the next menses rather than between 3 and 11, however, we found no inconsistencies when we used the slightly wider range. A serum P4 >3.0 ng/mL was considered as indicative of ovulation.

Statistical methods:

Modeling of the logarithm of serum E2 and P4 values was conducted with adjustment for days to start of the next menstrual cycle. Only ovulatory cycles were used in the analyses of serum hormone levels. Days to next cycle (from day 11 blood draw) is adjusted for as a linear variable for follicular E2. Days to next cycle (from blood draw that was used for ovulatory status determination and for luteal E2 and P4 values) is adjusted for as a linear and quadratic variable. These adjustments account for the known daily changes in serum E2 and P4 value during a normal menstrual cycle.

Results

Table 1 shows the Baseline and During Training results on ovulation frequency. At Baseline, 8 of the 53 women (15.1%) who had an informative blood sample, were anovular. At the During-Training cycle, 7 of the 53 women (13.2%) who had an informative blood sample were anovular.

Table 2 shows the results for serum E2 and P4 levels for the 38 women who ovulated during both the Baseline and During-Training cycles. It is necessary to restrict analyses to these matches cycles because of the known inter-individual variation in ovarian steroid levels.

No significant changes in follicular or luteal E2 levels were evident. Follicular E2 increased 0.5% and luteal E2 decreased by 0.3% - both compatible with, and strongly suggestive of, no changes in E2 levels.

SHBG was similarly unaffected, as was E2/SHBG, a measure of bioavailable E2.

The average P4 levels was, however, reduced During Training by 12.1%, although this difference is not statistically significant (1-sided p=0.10).

The average cycle length at Baseline for these 38 women who ovulated during both cycles was 29.8 days. At the During-Training cycle, their average cycle length was virtually unchanged at 29.5 days.

Conclusion

The moderate exercise program implemented in this study had no effects on ovulatory frequency, serum E2, bioavailable E2, or cycle length. It is possible that serum P4 levels were reduced (estimated reduction of 12.1%) but the reduction was not statistically significant (1-sided p=0.10).

Although such a reduction (12.1%) in serum P4 might appear of little importance, this is not necessarily so, as the effects of hormone changes have to be raised to the 4^{th} or 5^{th} power to see their effects on breast cancer risk [3].

This serum P4 result is based on a single P4 measurement during Baseline and a Single P4 measurement During Training, and is thus subject to much random error as suggested by the wide confidence limits on the observed 12.1% reduction in P4 (see Table 2). During these same cycles we collected daily urine cycles we collected daily urine samples. Urinary pregnanediol (Pdg; standardized to urinary creatinine) reflects serum P4, and by measuring Pdg at multiple times during the cycle (~10 times per cycle) we hope to be able to significantly reduce the random error in the comparison of progesterone levels and also reduce the number of indeterminate cycles. This is currently underway in Dr. Stanczyk's laboratory where he expects the assays (~58x2x10=1,160) to be completed in August.

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Table 1. Number of Study Subjects by Ovulatory Status[a]

	During Training			
	Anovular	Ovular	Indeterminate	Total
Baseline				
Anovular	4	4	0	8
Ovular	3	38	4	45
Indeterminate	0	4	1	5
Total	7	46	5	58

[a]Ovulatory status as determined by P4 level between 3 and 11 days before onset of next menses. 'Indeterminate' status indicates no appropriate serum sample available. During-Training cycle is after 4 to 6 months on the moderate exercise regimen.

Table 2. Baseline and During Training Serum Levels of Estradiol (E2) and Progesterone (P4) in Women on a Moderate Exercise Regimen[a]

	Baseline	During Training	Differences: ±95% CI
Follicular E2 (pg/mL)	141.0	143.6	+0.5% (-12.6%; 15.5%)[b]
Luteal E2 (pg/mL)	194.7	188.7	-0.3% (-8.2%; 8.9%)[c]
Luteal P4 (ng/mL)	15.4	14.1	-12.1% (-27.2%; +6.2%)[c]
Follicular SHBG (nmol/L)	44.2	43.6	-1.4% (-7.7%; +8.9%)[b]
Luteal SHBG (nmol/L)	48.5	50.7	+3.0% (-2.6%; +9.0%)[c]
Follicular E2/SHBG	3.41	3.34	+2.0% (-12.5%; +18.8%)[b]
Luteal E2/SHBG	4.54	4.06	-3.0% (-11.1%; +5.9%)[c]

[a]Women had to ovulate in both the Baseline and During-Training cycles to be included in this table. During-Training cycle is after 4 to 6 months on the moderate exercise regimen.
[b]Adjusted for days to next menses in the two cycles as linear terms.
[c]Adjusted for days to next menses in the two cycles as linear and quadratic terms.